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For the control of highly pathogenic avian influenza and preparedness for pandemic influenza

Hiroshi Kida, DVM, PhD, MJA
Professor, Graduate School of Veterinary Medicine
Head, Research Center for Zoonosis Control
Head, OIE Reference Laboratory for Avian Influenza
Head, WHO Collaborating Centre for Zoonoses Control
Hokkaido University, Sapporo, Japan
Questions for the control of HPAI and preparedness for pandemic flu

1. Why have the H5N1 HPAIVs persisted in poultry for 15 years and why have antigenic variants been selected in poultry birds?
2. Will the HPAIVs returned to migratory birds persist in nature?
3. How should HPAI be controlled just in poultry?
4. Does AI vaccine confer complete protective immunity?
5. Will H5N1 HPAIV cause pandemic influenza?
6. Are the measures for the control of seasonal flu satisfactory?

Answers should be given on the basis of the following findings:

Ecology of influenza viruses in nature, birds and mammals:
Origin, perpetuation in nature, and evolution of influenza viruses, and mechanisms of the emergence of HPAIV and pandemic influenza virus strains in humans.
Host range, and HA and NA subtypes of influenza A virus

- H1N1
- H1N2
- H3N2
- H2N3, H3N1, H3N3, H3N8, H4N6, H5N1, H5N2, H9N2

- H1N1
- H2N2
- H3N2
- (H2N8, H3N8)
- H5N1, H7N7, H9N2

- H3N8
- H7N7

- H1-H12
- N1-9

- H1-10
- N1-9

- H1-7, H9-16
- N1-9

- H1N3, H13N2, H13N9

- H3N2
- H5N1
- H10N4

- H3N3
- H4N5
- H7N7

- H1N3
- H13N2
- H13N9
Duck influenza

- Each of the known subtypes (H1-16, N1-9) of influenza A virus has been isolated from ducks.
- In ducks, viruses replicate in the colon, being shed with feces in a week, and non-pathogenic.
- Water-borne fecal-oral transmission.
- Ducks carry and provide nonpathogenic viruses during migration and over-wintering.
- Influenza viruses circulating in ducks are evolutionally stasis.

Migratory ducks are the natural host of influenza A viruses.

A/duck/S China/x/67 (H3NX)  
Domestic duck or goose  

Genetic reassortment  

Pig  
Cell  

A/S China/x/6/7 (H2N2)  

A/Hong Kong/1/68 (H3N2)  
Humans  

Virus shedding
Genetic reassortment
A/HongKong/68 (H3N2)
A/Asian/67 (H2N2)
A/duck/ xx (H3N?)
Domestic duck
Pond
Virus shedding
Genetic reassortment
1968 HK
A/HongKong/68 (H3N2)
1918 Spanish virus (H1N1)
1957 Asian virus (H2N2) and even 1918 Spanish flu virus (H1N1) must have appeared similarly.

Rout of transmission of the genes of pandemic strains
The role of pigs in the emergence of pandemic influenza virus strains

- Pigs are susceptible to infection with avian influenza viruses of each of the HA subtypes.
- Genetic reassortants were generated in the cells lining upper respiratory tract of pig upon concurrent infection with mammalian and avian strains.

Candidates of future pandemic influenza virus strain

- H1 to H16 and N1 to N9 subtypes of influenza A viruses perpetuate in the lakes where ducks nest in summer.
- 1957 H2N2, 1968 H3N2, and even 1918 H1N1 pandemic influenza viruses are reassortants between AIV and human strains.
- Pigs are susceptible to both of avian and mammalian viruses, generating reassortants on the concurrent infection.

→ Avian viruses of any subtype can contribute genes for reassortants. Therefore, none of the 16 HA and 9 NA subtypes can be ruled out as potential candidates for future pandemics.

→ Global surveillance of swine flu as well as avian flu is important.
Acquisition of pathogenicity of avian influenza viruses in chickens

APAIV

LPAIV

6~9 Months

HPAIV (H5 or H7)
Acquisition of pathogenicity of avian influenza viruses in chickens and
Return of the HPAIV from domestic poultry to migratory water birds

Highly pathogenic AIV (H5 or H7)

Non-pathogenic AIV

Low pathogenic AIV

> 6 months

Highly pathogenic AIV (H5 or H7)
HPAI viruses isolated from wild birds in Mongolia

- A/whooper swan/Mongolia/3/05 (H5N1)
- A/bar-headed goose/Mongolia/1/05 (H5N1)
- A/common goldeneye/Mongolia/12/06 (H5N1)
- A/whooper swan/Mongolia/2/06 (H5N1)
- A/whooper swan/Mongolia/2/09 (H5N1)
- A/whooper swan/Mongolia/9/09 (H5N1)
- A/bar-headed goose/Mongolia/X53/09 (H5N1)
- A/rubby sholduck/Mongolia/X42/2009 (H5N1)
- A/common goldeneye/Mongolia/X60/09 (H5N1)
- A/whooper swan/Mongolia/1/10 (H5N1)
- A/whooper swan/Mongolia/7/10 (H5N1)
62 Countries where H5N1 HPAIV infections were reported in wild birds, poultry, and both
Japan, Republic of Korea, China, Mongolia, Myanmar, Lao PDR, Thailand, Cambodia, Viet Nam, Malaysia, Indonesia, Bangladesh, India, Pakistan; Afghanistan, Iran, Azerbaijan, Georgia, Iraq, Kuwait, Saudi Arabia, Turkey, Israel; Russian Federation, Kazakhstan, Ukraine, Romania, Bulgaria, Albania, Serbia, Hungary, Slovakia, Czech Republic, Croatia, Poland, Slovenia, Bosnia & Herzegovina; Greece, Switzerland, Austria, France, Italy, Germany, Netherlands, Denmark, Sweden, Spain, England, Ireland; Djibouti, Gaza Strip, Egypt, Sudan, Nigeria, Niger, Cameroon, Burkina Faso, Cote d’Ivoire
Confirmed human cases of H5N1 HPAIV infection

<table>
<thead>
<tr>
<th>Country</th>
<th>Deaths/Cases</th>
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<tbody>
<tr>
<td>China</td>
<td>28 / 42</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>61 / 123</td>
</tr>
<tr>
<td>Indonesia</td>
<td>156 / 188</td>
</tr>
<tr>
<td>Egypt</td>
<td>60 / 167</td>
</tr>
<tr>
<td>Cambodia</td>
<td>17 / 19</td>
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<tr>
<td>Lao PDR</td>
<td>2 / 2</td>
</tr>
<tr>
<td>Thailand</td>
<td>17 / 25</td>
</tr>
<tr>
<td>Iraq</td>
<td>2 / 3</td>
</tr>
<tr>
<td>Azerbaijan</td>
<td>5 / 8</td>
</tr>
<tr>
<td>Turkey</td>
<td>4 / 12</td>
</tr>
<tr>
<td>Djibouti</td>
<td>0 / 1</td>
</tr>
<tr>
<td>Nigeria</td>
<td>1 / 1</td>
</tr>
<tr>
<td>Myanmar</td>
<td>0 / 1</td>
</tr>
<tr>
<td>Pakistan</td>
<td>1 / 3</td>
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<tr>
<td>Bangladesh</td>
<td>0 / 3</td>
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</tbody>
</table>

Total 355 / 602

As of 12 April 2012

WHO (2012), Kuribayashi
Bird flu vaccines

**Vietnam:**
H5N2 and H5N1
(Adjuvant inactivated vaccines)

**China:**
H5N1 and recombinant NDV
(Reverse genetics inactivated vaccines)

**Indonesia:**
H5N1, H5N2, H5N9 and recombinant H5N1
(inactivated vaccines)

**Egypt:** since 2006

**Thailand:**
Officially prohibited vaccination in 2006

As a stockpile,

**Singapore:**
H5N2
(Inactivated, adjuvanted vaccine)

**Japan:**
H5N1 and H7N7
(Oil-adjuvanted inactivated vaccines)

**Pakistan:**
H5N1, H5N2, H5N9, and H5N3
(Water based with alum hydroxide and oil based with mineral oil)
Influenza Vaccine for bird flu

- may prevent manifestation of disease signs and decrease the amount of virus shed, but does not confer protective immunity from infection.

- “Stamping-out policy”, including early detection, compensation, culling of the flock, and strengthening hygienic measures and monitoring, is recommended for the control of avian influenza.

- Vaccination was not primarily recommended but later approved as one of the options applied under the stamping-out strategy.

- Country where vaccine is used is not designated as HPAI-free.

→ leads silent spread of virus.
RECOMMENDATION FOR THE CONTROL OF AVIAN INFLUENZA

It is considered that;

- Highly pathogenic avian influenza H5N1 virus strains have persisted in domestic poultry for 14 years and antigenic variants have been selected mainly due to the misuse of vaccine.
- HPAI has been put under control in several countries.
- Stamping out policy has been the most effective measures for the control HPAI.
- Vaccine is used in 4 countries where HPAI has not been controlled yet.
- Vaccine is used instead of stamping out in 2 countries and in the other 2 countries, basically in addition to stamping out.
- Sentinel bids are put in the vaccinated poultry population in Viet Nam and not in the other 3 countries where vaccine is used.
- Compensation for livestock owners is done in most countries in case of stamping out.

It is recommended that;

1. Since stamping out is the best and ultimate measure for the control of HPAI, vaccine should be used in addition to, not instead of stamping out.
2. The OIE should continue and develop standards on animal influenza surveillance, prevention and control.
3. Surveillance of swine flu is crucial in the countries where avian flu has not been controlled.

Mongolia (324)
- H1N1 (5)
- H2N3 (1)
- H3N6 (34)
- H4N2 (2)
- H4N6 (72)
- H5N3 (4)
- H7N6 (1)
- H7N9 (3)
- H9N2 (1)
- H10N5 (2)
- H12N5 (1)

H2N2 (1)
H3N2 (149)
H4N3 (1)
H4N7 (1)
H5N2 (1)
H7N1 (2)
H7N7 (13)
H8N4 (6)
H10N3 (13)
H10N7 (5)

Australia (6)
- H2N3 (1)
- H3N8 (1)
- H4N6 (1)

China (2)
- H3N8 (1)
- H4N6 (1)

Russia (56)
- H3N8 (17)
- H4N9 (2)
- H11N1 (1)
- H11N9 (8)

H4N6 (25)
H10N7 (1)
H11N6 (1)
H13N6 (1)

Hokkaido (296)
- H1N1 (12)
- H2N5 (1)
- H3N8 (37)
- H4N6 (33)
- H5N3 (11)
- H6N5 (2)
- H7N1 (18)
- H9N2 (7)
- H9N9 (1)
- H10N5 (7)
- H10N8 (1)
- H11N9 (21)
- H13N6 (2)

H2N2 (2)
H3N2 (2)
H3N6 (6)
H4N9 (3)
H5N2 (1)
H6N1 (17)
H6N8 (7)
H7N7 (14)
H9N4 (1)
H10N2 (1)
H12N2 (2)
H5N1(1)

H4N6 (2)
H10N7 (1)
H11N6 (1)
H13N6 (1)

USA (111)
- H2N3 (1)
- H3N8 (39)
- H4N6 (57)
- H7N7 (1)
- H8N2 (1)
- H10N7 (11)

H5N1 (1)
H11N1 (1)
H11N6 (1)
H13N6 (1)

H10N6 (1)
H10N7 (12)
H11N6 (1)
H12N5 (4)

795 isolates from 22,744 samples
Surveillance of avian influenza in autumn 2010

Mongolia (36 isolates)
- H1N1 (1)
- H3N3 (1)
- H3N6 (7)
- H3N8 (14)
- H4N6 (8)
- H7N9 (1)
- H10N8 (4)

Hokkaido (15 isolates)
- H3N8 (3)
- H5N2 (1)
- H6N2 (2)
- H7N7 (9)
- H5N1 (2)

Vietnam (1 isolate)
- H9N6 (1)

Hong Kong (3 isolates)
- H3N2 (1)
- H5N1 (2)

Laos (none)

Number of samples: 4,515
Influenza virus isolates: 55
As of 25 October 2010
Time line of H5N1 HPAIV infection outbreaks in Japan 2010 - 2011

Ito T (2011)
Phylogenetic analysis of the influenza virus isolates (clade 2.3.2)

Okamatsu (2011)
Outbreaks of HPAI caused by H5N1 viruses in Japan in 2010–2011 winter

Wild birds (63)
Chicken farms (24)

Okamatsu (2011)
HPAI virus and human pandemic virus strains
Thus, 246 avian influenza viruses of 144 combinations of HA and NA subtypes have been stocked as vaccine strain candidates. Their pathogenicity, antigenicity, genetic information and yield in chicken embryo have been analyzed, databased ([http://virusdb.czc.hokudai.ac.jp/vdbportal/view/index.jsp](http://virusdb.czc.hokudai.ac.jp/vdbportal/view/index.jsp)).

<table>
<thead>
<tr>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>N6</th>
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</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>H2</td>
<td>H3</td>
<td>H4</td>
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<td>H6</td>
<td>H7</td>
<td>H8</td>
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Influenza viruses of 69 combinations of the HA and NA subtypes have been isolated from fecal samples of ducks in Alaska, Siberia, Mongolia, Taiwan, China, and Japan (black).

75 combinations have been generated by genetic reassortment procedure in the laboratory (red).

Isolates from water birds (69 combinations) | Reassortants generated in the lab (75 combinations)

Thus, 246 avian influenza viruses of 144 combinations of HA and NA subtypes have been stocked as vaccine strain candidates. Their pathogenicity, antigenicity, genetic information and yield in chicken embryo have been analyzed, databased ([http://virusdb.czc.hokudai.ac.jp/vdbportal/view/index.jsp](http://virusdb.czc.hokudai.ac.jp/vdbportal/view/index.jsp)).
For the control of HPAI and preparedness for the next pandemic influenza

1. Why have the H5N1 HPAIVs persisted in poultry for 15 years and been antigenic variants selected? Misuse of Vaccine.

2. Will the HPAIVs returned to migratory birds persist in nature?
   Started contamination of HPAIVs in the nesting lakes of migratory ducks. Eradication of the H5N1 HPAIVs from poultry throughout the world is urgently needed.

3. How should avian influenza be controlled in poultry?
   Stamping-out policy, including enhanced surveillance, early detection, culling the flock, movement restriction, and monitoring without misuse of vaccine, is the ultimate standard.

4. What are the advantage and disadvantage of the use of vaccines?
   Vaccine should be carefully used in addition to, not instead of stamping-out.

5. Will H5N1 HPAIV cause pandemic influenza?
   It is unlikely to occur that direct transmission of AIV from birds to humans, but may occur via pigs. H5N1 alone is not a candidate of pandemic strain.

6. Are the measures for the control of seasonal flu satisfactory?
   How to control pandemic influenza should be based on the measures for the control of seasonal influenza.

★ Global surveillance of avian, swine and human influenza, and seasonal flu control measure-based strategy should be intensively carried out by international collaboration under the umbrella of One World One Health concept.
Research and Development Model for Zoonosis Control

Vaccine strain candidates

Molecular basis of pathogenesis

Interspecies transmission

Panel of antisera

Prediction of antigenic drift

Global surveillance

Prediction of pandemic influenza virus subtypes

Mucosal vaccine

Isolate from ducks

Genetic reassortant

Antivirals

Global surveillance

North America
Zoonoses should be controlled when the novel field of science is established by the collaboration with and fusion of Veterinary Medicine, Medicine, Public Health, Ecology, Epidemiology, and Computer Science.
We must accept the fact that zoonoses are not eradicable infections since most of the causative pathogens are introduced from wildlife in nature. Such zoonotic infections, therefore, can be controlled only by taking preemptive measures to predict and prevent their outbreaks.

For the establishment of preemptive measures against zoonoses, a prerequisite is to identify natural host animals carrying potential pathogens and to elucidate the transmission routes and factors involved in the spread and pathogenesis of infections. As well as promoting basic research on zoonotic diseases, there is a pressing need to develop effective measures for diagnosis, prophylaxis and therapy, to widely disseminate information and technology, and to train experts for the control of zoonoses.

Hokkaido University Research Center for Zoonosis Control, thus, carry out coherent scientific and educational activities for the control of zoonoses.